

# Using metabolic networks to resolve ecological properties of microbiomes

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## Abstract

The systematic collection, integration and modelling of high-throughput molecular data (multi-omics) allows the detailed characterisation of microbiomes *in situ*. Through metabolic trait inference, metabolic network reconstruction and modelling, we are now able to define ecological interactions based on metabolic exchanges, identify keystone genes, functions and species, and resolve ecological niches of constituent microbial populations. The resulting knowledge provides detailed information on ecosystem functioning. However, as microbial communities are dynamic in nature the field needs to move towards the integration of time- and space-resolved multi-omic data along with detailed environmental information to fully harness the power of community- and population-level metabolic network modelling. Such approaches will be fundamental for future targeted management strategies with wide-ranging applications in biotechnology and biomedicine.

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## Keywords

Ecological interactions, Keystone gene, Function and species, Metabolic network and model, Microbial systems ecology, Niche breadth.

## Microbial systems ecology

Microbial communities (microbiomes) are involved in all biogeochemical cycles by contributing functions which may be common to most ecosystems (underlined words are defined in [Box 1](#)), e.g. nitrogen fixation, or by being first-line to very specific ecosystem services, e.g. the degradation of particular xenobiotics. Although the global relevance of microbial activities for ecosystem functioning is now widely accepted, methods to study the ecology of the tremendous richness of the microbial realm are relatively recent. In order to model, predict and understand the behaviour of microbial constituents in their native environments, Microbial Systems Ecology heavily relies on high-throughput, high-fidelity and high-resolution measurements of microbial consortia ([Figure 1A](#)) as well as the integration of the resulting data [[1](#)]. Thereby, Microbial Systems Ecology relies on specialised wet- and dry-lab approaches to achieve coherent assessments of microbial community structure and function *in situ* [[1–5](#)]. In addition to the valuable insights on community structure and functional potential (metagenomics), expressed functions (metatranscriptomics and metaproteomics) and metabolic activity (metabolomics), the integration of the individual omic levels ([Figure 1B](#)) allows comprehensive resolution of the emergent properties of ecosystems [[1,6](#)]. Furthermore, integrative approaches can significantly reduce the current limitations associated with single omics by enhancing the interpretability of data [[1](#)], allowing for example to obtain improved genome reconstructions from constituent populations [[7](#)] and to link the expression of phenotype-associated microbial functions to distinct taxa [[8](#)].

Natural microbial communities are comprised of constituent, interacting populations. Therefore, to move from descriptive, comparative or statistical studies to ecological inferences [[9](#)], in Microbial Systems Ecology, microbial communities must be seen as networks of networks: community members (populations), consisting of collections of interwoven molecular networks, form the interacting units of higher-order ecological systems. Although different types of molecular networks exist (e.g. gene regulatory networks, co-occurrence networks, etc.), we particularly focus our review on metabolic network reconstruction and related modelling approaches as applied to microbial communities in view of resolving specific properties underpinning ecosystem functioning. We also present our opinion on how harnessing this ecological knowledge will facilitate

**Box 1. Glossary**

- Ecosystem: ecological self-supporting unit constituted of an environment (the biotope) and the living organisms inhabiting it (the biocoenosis). Despite flows of materials, organisms and energy occurring across the boundary of individual units, the two components of an ecosystem interact more strongly between each other than with the neighbouring units.
- Ecosystem functioning: all activities, processes and properties driving biogeochemical activities and leading to the relative ecological stability of an ecosystem.
- Ecological niche (Hutchinson): the hypervolume comprised of  $n$  dimensions representing the environmental conditions and resources gradients enabling a species to persist. This definition led to the subsequent description of the fundamental niche (the maximal usable space) and the realised niche (the actual used space).
- Ecological interactions (or biological interactions or symbiosis): long-term relationship between individuals of different species including mutualism (win–win), commensalism (win–neutral), parasitism/predation (win–lose) and amensalism (lose–neutral). Metabolic interactions represent a subset of these relationships when the interaction is mediated through one or multiple metabolite(s), as opposed to non-metabolic relationships.
- Metabolic models: *in silico* description of the metabolic potential of a biological unit (e.g. community, guild, species), often represented as a bipartite directed network consisting of metabolites and reactions/enzymes/genes [12]. While topological metabolic models represent a qualitative view of metabolism, stoichiometric metabolic models require the specification of each reaction's stoichiometry in a stoichiometric matrix, which forms the basis for quantitative metabolic modelling.
- Microbial Systems Ecology: the holistic study of microbial communities using systems biology approaches.
- Systematic measurement: “the standardised, reproducible, and simultaneous measurement of multiple features from a single sample. Resulting datasets are fully integrable and relate system-wide behaviours” [1].

targeted manipulations of microbial communities in the future. More specifically, space- and time-resolved integrated multi-omic datasets will allow us to define and subsequently alter the realised niches of constituent populations for the management of community–conferred traits.

## Using metabolic networks to obtain meaningful ecological insights

### Reconstruction, analysis and modelling of metabolic networks

Community-level metabolic modelling approaches are classified according to the unit being modelled (entire community, guilds, species or strains, see Figure 1B and C) [10] and the level of detail. Metabolic modelling approaches may be divided into i) stoichiometric approaches that model the metabolism quantitatively [11], and ii) topological (network-based) approaches, which are more suitable for qualitative metabolic modelling [12].

In any case, a prerequisite to metabolic modelling is metabolic network reconstruction, i.e. the assembly of a

metabolic map for the unit of interest. A number of automatic pipelines generate metabolic reconstructions directly from the genome [13–15] or metagenome [16], which can subsequently serve as the starting point for manual curation [17]. Alternatively, a selected subset of pathways relevant in a particular environment can be targeted for metabolic reconstruction [18]. Two major challenges for metabolic reconstruction are i) the large number of genes without functional annotation, which can be partially overcome using gap filling methods [19], and ii) the association of genes to reactions. Semi-curated metabolic models are collected in repositories such as AGORA [20].

Once a metabolic network reconstruction has been obtained, the community's metabolism can be analysed qualitatively or quantitatively. For instance, a topological analysis can serve to identify specific metabolic pathways of interest or to extract the active part of a community's metabolism from metatranscriptomic [21], meta-proteomic or (meta-)metabolomic data (Figure 1B). A widespread quantitative metabolic modelling approach is flux balance analysis (FBA), which calculates the metabolite flow through reactions such that a particular objective function, e.g. biomass production, is maximised [11]. While topological metabolic models can integrate omics data via node or edge weights, stoichiometric models can take them into account for instance by modifying flux distributions [22]. FBA, which was originally developed for single species, was recently extended to multiple species [23,24]. However, these approaches only provide a static picture of the community. Dynamic community-level metabolic modelling, which describes the change of species abundances and metabolite concentrations over time, currently is an active field of development [25,26].

In the following paragraphs, we will discuss some applications of metabolic modelling in more detail, namely the prediction of ecological interactions, identification of keystone species and functions as well as metabolic niche inference.

### Metabolic interactions

Metabolic models can be exploited to predict ecological interactions between species via metabolic cross-feeding, for instance in the case of mutualistic growth on the toxic end-products of other species, or when two species compete for the same nutrients (Figure 1C and D). Importantly, the extracellular environment, which can be characterised by metabolomics and physicochemical measurements, needs to be taken into account when predicting interactions, since not all potential interactions will be actually realised particularly in nutrient-rich environments [27]. A number of stoichiometric interaction prediction approaches compare growth rates computed in the presence or the absence of

an interaction partner [28–30] or under different environmental condition [31] to determine the interaction type. Here, COMETS [26] also takes into account the impact of spatial structure on cross-feeding.

In contrast to analyses based on stoichiometric modelling, topology-based interaction prediction [32–34] first involves the inference of seed metabolites for a given microbial population, which include all metabolites that cannot be produced by the network itself [35]. It then assesses whether some of these seeds can be produced by the metabolic network of another species, which in turn allows quantification of the potential for commensalism or mutualism. The metabolic interaction potential measures the maximum number of essential nutrients that an organism can obtain by interacting with its community [34]. Furthermore, the competitive potential between two species can be determined by computing the overlap between their seed metabolites [36].

An alternative topological approach finds genome segments that maximise the number of consecutive enzyme-coding genes. The enzymes in turn catalyse metabolic transformations which are complementary across species [37]. Metabolic pathway complementarity or overlap can also be exploited to screen metagenomic data for interactions. This form of topological analysis has for instance been applied to explore metabolic strategies in human gut microbiota [38].

Recent work has involved the use of multi-omics to refine or validate model predictions in different environmental conditions [39–42]. Beyond interactions mediated through exchange or competition for metabolites, trophic interactions such as phage predation can also be inferred using omic data (see Box 2 for an example of non-metabolic interactions). Similarly, additional ecological insights such as keystone roles of some species can be inferred when metabolic networks are combined with other layers of knowledge such as co-occurrence of genes/transcripts/proteins/metabolites or to regression- and rule-based network analysis [43].

### Keystone functions, genes and species

Ecological keystone species are commonly understood as species that have a pronounced impact on their environment independent of their abundance, i.e. they have a disproportionate deleterious effect on the community upon their removal [44,45]. This concept reflects the dependencies within a community governed by interactions among its members and is clearly context-dependent: the importance of any organism for stabilising the community is conferred by the particular group. Thus being a keystone species is not a Boolean trait, but it is rather a continuous property that emerges in the context of community function and different selection pressures. In order to predict which organism is a functional keystone species, the topological properties of

networks derived from metabolic models that represent the community-wide organisation of microbial interactions may be used (Figure 1E) in synergy with co-occurrence networks [46,47]. Measures such as degree, clustering coefficient and closeness centrality reflect the scale of the embeddedness of the constituting organisms (nodes) in the microbial community ranging from direct ecological partners to local and global neighbourhoods, respectively [46].

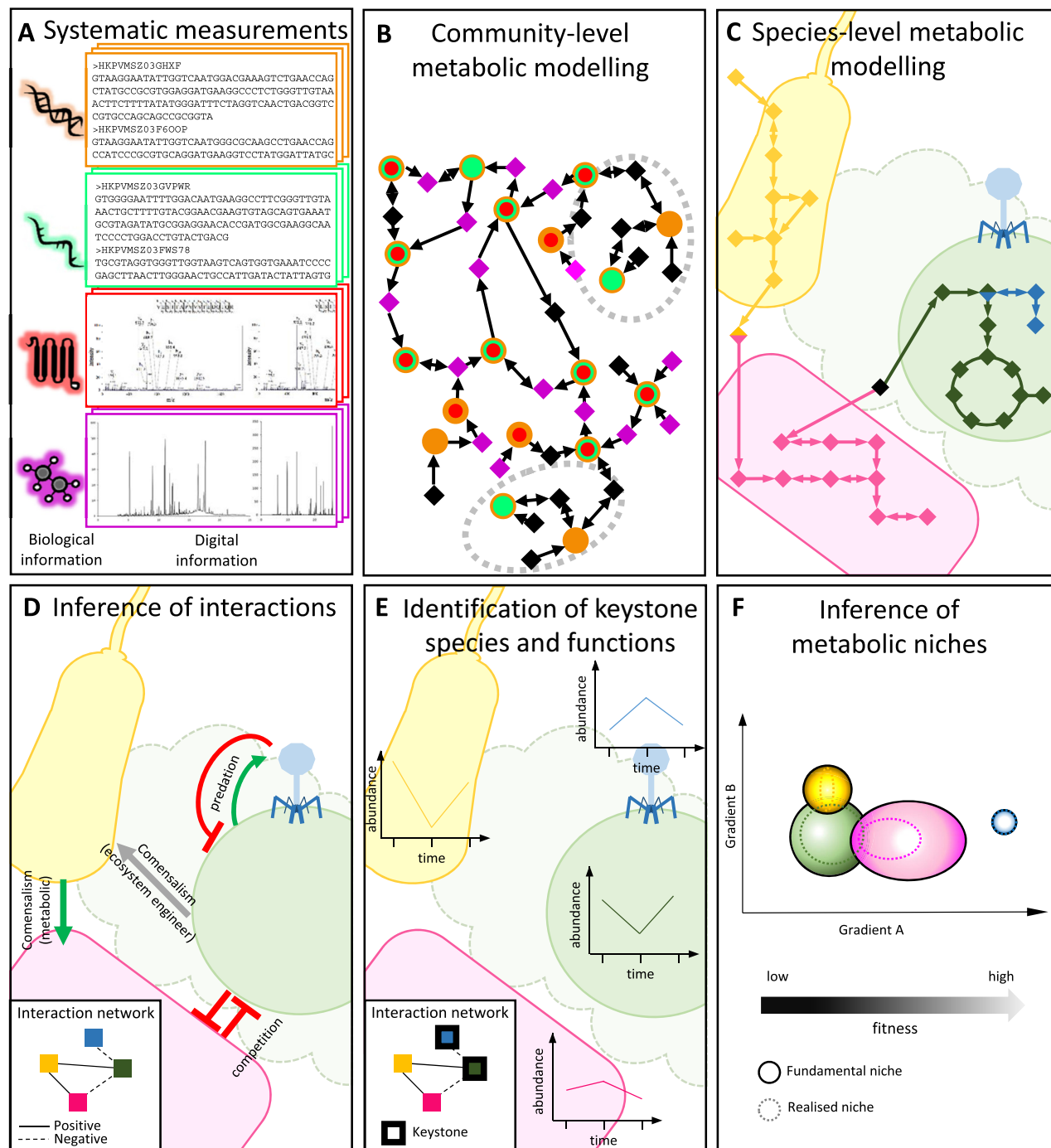
Different categories of keystone species have been proposed including ecosystem engineer (or modifier) keystone species (Figure 1E), trophic (prey or predator) keystone species or resource provider keystone species [48]. In any case, keystone species confer keystone functionalities to the ecosystem [49]. For example, the degradation of dietary fibres in the human gut is the result of a community-driven effort. However, the pivotal step is the breakdown of the complex resistant starches like amylopectin and amylose by primary degraders, which release simple sugar molecules to be fermented by the rest of the microbial consortium. *Ruminococcus bromii* is a keystone species in this context [50]. The organism possesses a highly specific cluster of keystone genes essential for efficient amyolysis [51].

Keystone metabolic genes are predicted to be highly expressed despite typically low gene copy numbers (reflecting the typical relatively low abundance of keystone species) and to catalyse key biochemical transformations (enzymes represent “load points” in the community-wide metabolic networks [52]). Therefore, a framework has been developed for the identification of such genes in reconstructed community-wide metabolic networks [49]. High relative gene expression (extracted from metatranscriptomic and/or metaproteomic data relative to gene abundance information derived from the corresponding metagenomic data) as well as specific network topological features (low relative degree and high betweenness centrality) are taken into account for the identification of such keystone genes which, through genomic linkage to reconstructed population-level genomes, can be linked to specific constituent populations which represent keystone species [49]. This approach has highlighted ammonia monooxygenase as a keystone gene in a biological wastewater treatment plant which is contributed to the community function by a specific keystone strain of *Nitrosomonas* spp [49]. Community-wide reconstructed metabolic networks are thereby particularly informative for the identification of keystone traits conferred by specific keystone species.

### Microbial niche ecology

Even though it has been shown that clusters in a co-occurrence network based on 16S rRNA sequencing data reflect overlapping ecological niche preferences and common habitats of populations [53], the inference of niches of distinct bacterial populations in microbial

Figure 1



From metabolic models to ecological insights. (A) Following carefully adapted wet-lab procedures and systematic measurements of the purified bio-molecules, (B) metabolic modelling (here resolved to the community level) by stepwise integration and modelling of the metagenomic (blue), meta-transcriptomic (green), metaproteomic (red) and (meta-)metabolomic (pink) data, allows to detect, for example, parts of the metabolic network that are inactive (dotted line circle) at the sample collection. (C) Metabolic modelling (here resolved to the species level), often represented as a directed network consisting of metabolites (nodes) and reactions (edges), can be a starting point to determine (D) an ecological interaction network (nodes = species; edges = interactions). Although some non-metabolic interactions, such as commensalism by niche engineering (e.g. the green organism is a biofilm founder, allowing a secondary colonisation by the yellow microbe) or predation (see Box 2) cannot be predicted from inferred metabolic networks, other complementary analyses, such as co-occurrence networks, will allow to predict such behaviour. (E) Topological analysis of metabolic, interaction and co-occurrence networks allow the detection of metabolic keystone species (highlighted in green; bacterial species) and trophic keystone species (highlighted in blue; phage). (F) The use of population-resolved metagenomic data to describe the fundamental niche is extended by the use of functional omic data to characterise the realised niche of different species. From this information, predictions can be made for example in relation to the fitness gradients of constituent populations.



**Box 2. Causal inference of non-metabolic interactions (i.e. phage-host interactions from metagenomic data)**

Phages are the most abundant and diverse entities in any environment, greatly influencing microbial community structure and dynamics through affecting the prokaryotic (host) metabolism [68,69], modulating nutrient cycles, and driving long-term host evolution [70]. The unculturability of the vast majority of host and phage strains can be circumvented by integrating meta-omic data [71,72]. Accordingly, computational methods have been developed to identify phages [73,74] and predict links to their putative hosts [75].

In addition, time-resolved datasets enable the inference of phage-host dynamics [76,77], which will result in improved knowledge and, thereby, the formulation of potential phage treatment strategies for biomedical and biotechnological applications [78].

communities remains a challenging task, due to the inherent complexity of trophic interactions and fluctuating environmental conditions. In that sense, integrated multi-omic approaches have been shown to be useful for studying microbial niche ecology. State-of-the-art binning approaches [54], or ensemble methods [55], allow near complete reconstruction of population-level genomes from assembled sequencing reads. By applying the traditional concepts of niche ecology by Hutchinson, the genomic functional potential of a microbial population reflects its fundamental niche [56,57]. Conversely, metatranscriptomic or metaproteomic data can be used to infer a population's realised niche at the time of sampling [57], while intra- and extracellular metabolomic data allows inferences regarding resource usage and the overall resource space available, respectively [57] (Figure 1F). Previous studies have relied on gene expression patterns to assess life-style strategies (generalists versus specialists) and the metabolic niche breadth of distinct populations [57,58]. Computational approaches that automatically predict phenotypic traits of reconstructed genomes [59] are an important resource for the in-depth characterisation of niche occupation. In this context, metabolic models can provide a detailed picture on growth conditions, such as available carbon or nitrogen sources and models have indeed been used to predict medium requirements reflecting niche breadths [60].

Apart from resource availability and usage, niche breadth also reflects tolerance ranges to physico-chemical variables, such as pH, temperature or dissolved oxygen, which are generally available only for cultured isolates. Currently, a popular approach involves the linking of inferred organismal abundances to environmental conditions, which can be challenging due to the compositional nature of rRNA amplicon sequencing data. Leveraging integrated multi-omic data and metabolic models may in turn provide a detailed mechanistic understanding of the adaptation to environmental factors

for single organismal groups, as demonstrated for pH-dependent metabolic adaptations of *Enterococcus faecalis* [61].

**Harnessing the power of data integration in Microbial Systems Ecology**

The integration, contextualisation and analysis of multi-omic data using metabolic network approaches (in synergy with other network approaches) offer many exciting opportunities in the context of Microbial Systems Ecology, a few of which are highlighted above. While such tools are commonly used in systems biology [62], their utilisation in (microbial) ecology is still limited.

In order to move beyond associations and hypotheses derived from integrated multi-omic data, model predictions will have to be tested using combinations of detailed field and/or laboratory experiments [1,5,63], as described for example in Ref. [64]. A discovery-driven planning approach, wherein systematic measurements, data integration, model generation, hypothesis testing and new ecological hypotheses follow each other iteratively, should culminate in predictive models [1]. Thus, system-wide data has to be collected in a manner consistent with the subsequent integration and modelling to continuously improve the community models; ultimately we aim for models which allow the systematic and knowledge-guided control of different microbial community functions and/or structures. In this context, keystone functions, genes and species represent primary targets for community management, because of their disproportionate effect on ecosystem functioning. For example, lipid accumulating organisms present in wastewater treatment plants are an abundant source of lipids which may be directly converted into biodiesel [65], but as the community phenotype shows seasonal fluctuations, economical interest remains limited. Bio-stimulation of endogenous keystone species(s) or targeted activation of keystone gene(s) would help tune the community towards the desired phenotype robustly around the year [63]. Conversely, a targeted removal of keystone functions may provoke a collapse of the community. In this context, the keystone concept was successfully used for the prediction of drug targets that control the pathological lung microbiome of persons with cystic fibrosis [66].

In the future, by determining the respective ecological niches of the constituent populations, we will be able to move beyond 'basic' ecological classifications of lifestyle strategy for microbes such as generalists and specialists towards more specific classifications such as the Universal Adaptive Strategy Theory (UAST) describing trade-offs between ruderal, stress tolerant and competitor behaviours [67]. This will further enable us to determine the metabolic basis of colonisation/immigration, successional stages and the community response to perturbations. In

order to establish such concepts, the field needs to move towards the integration of time- and space-resolved multi-omic data to unravel the functional dynamics of complex microbial communities. In our opinion, the elucidation of networks requires such longitudinal data and corresponding time-series analyses to model the populations' interplay as well as to highlight which parts of these networks are active under specific conditions. Hence, future augmented community-level metabolic models need to account for trophic interactions and changing environmental conditions, ideally by integrating dynamic community models with genome-scale metabolic models. Therefore, within the framework of Microbial Systems Ecology, we will in the future be able to systematically define and alter the realised niches of constituent populations *in situ* and manage community-conferred traits, leading to exciting prospects for biotechnology and biomedicine.

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